
Structural Insights into Sigma1 Function

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Contents

1	Introduction	14
2	Crystal Structure of Human Sigma1	15
3	Structural Basis for Ligand Recognition	17
4	Oligomerization	19
5	Implications for Sigma1 Function	20
6	Outlook	21
	6.1 Relationship to Erg2p and Enzymatic Activity	21
	6.2 Molecular Efficacy and Oligomerization	22
	6.3 Interactions with Other Proteins	22
	References	23

Abstract

Sigma1 (also known as this sigma-1 receptor) is an unusual and enigmatic transmembrane protein implicated in a diverse array of biological processes ranging from neurodegenerative disease to cancer. Despite decades of research, the molecular architecture of Sigma1 is only beginning to become clear. Recent work has established that Sigma1 is an oligomer, and crystallographic studies have now offered the first high-resolution views of its molecular structure. For the first time, these results provide a detailed framework to understand mutagenesis data and the molecular pharmacology of Sigma1 ligands. Structural data also raise new questions surrounding the mechanisms of ligand activity and the molecular basis for interactions between Sigma1 and other proteins. As Sigma1 research enters the structural era, the field is poised for new discoveries and reevaluation of old data and old models.

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13

Keywords

Crystal structure • Ligand binding • Oligomer • Sigma1 • Sigma-1 receptor • X-ray crystallography

1 Introduction

Sigma1 (also known as the sigma-1 receptor) is an unusual transmembrane receptor first discovered 40 years ago and subsequently studied extensively for its pharmacological properties, which in some respects resemble those of opioid receptors (Walker et al. 1990). However, the development of photoaffinity probes and enantiomerically pure ligands later showed that Sigma1 resemblance to opioid receptors is largely superficial. Unlike opioid receptors, Sigma1 has a molecular weight of ~25 kDa, and it shows a strong preference for the (+) enantiomers of benzomorphan ligands. In contrast, true opioid receptors selectively bind the (–) enantiomer (Martin et al. 1984). Moreover, the effects of Sigma1 ligands are not antagonized by the opioid antagonists naltrexone and naloxone (Largent et al. 1987), which block agonist activity at the μ , δ , and κ opioid receptors. Finally, unlike the opioid receptors, Sigma1 does not appear to signal through heterotrimeric G proteins (Lupardus et al. 2000), further confirming its lack of relation to opioid receptors.

While Sigma1 was extensively characterized with respect to its pharmacology in the 1980s and early 1990s, the first structural insights regarding Sigma1 came only in 1996 when the protein was cloned from guinea pig liver (Hanner et al. 1996), and shortly thereafter from a human cell line (Kekuda et al. 1996) as well as mouse (Seth et al. 1997) and rat tissues (Seth et al. 1998). Remarkably, the sequence of Sigma1 shows no similarity to any other mammalian protein, but it possesses clear homology (30% sequence identity) to the yeast C8–C7 sterol isomerase Erg2p. Hydrophobicity analysis of the guinea pig Sigma1 protein sequence showed a single highly hydrophobic segment with a potential ER-retention signal at its amino terminus, suggesting a single-pass transmembrane topology with a short luminal tail at the amino-terminus and the majority of the protein located on the cytosolic side of the ER membrane. As discussed below, this single-pass transmembrane model was less widely accepted than later proposed architectures placing both the amino- and carboxy-termini in the ER lumen, although the prediction of a single-pass fold was ultimately shown to be correct.

The determination of the receptor's primary sequence led to extensive prediction and speculation regarding the three-dimensional structure of Sigma1. One report (Aydar et al. 2002) proposed a two-pass transmembrane architecture on the basis of antibody staining with Sigma1 fused to amino- and carboxy-terminal green fluorescent protein (GFP). An important caveat to this work, however, is that GFP is known to be poorly secreted (Li et al. 2002), and the amino-terminal fusion may have altered the insertion topology of the protein. The two-pass model was widely accepted as established fact, serving as the basis for efforts to map the position of the putative second transmembrane domain (Ortega-Roldan et al. 2015) and other

studies using molecular modeling to predict the receptor's structure computationally (Laurini et al. 2011). In contrast, efforts to determine the Sigma1 structure experimentally lagged behind, hindered by the myriad challenges associated with biochemical manipulation of integral membrane proteins.

2 Crystal Structure of Human Sigma1

Beginning in 2007, a series of technological innovations revolutionized membrane protein crystallography, particularly for G protein-coupled receptors (GPCRs). Advances include the use of fusion proteins like T4 lysozyme (Rosenbaum et al. 2007), lipid mesophase crystallization techniques (Caffrey et al. 2012), microdiffraction (Smith et al. 2012), and novel detergents (Chae et al. 2010). Together, these methods have led to dozens of GPCR structures, and these approaches are increasingly finding application in membrane protein crystallography more generally.

To determine the structure of Sigma1, a GPCR-inspired approach was employed (Schmidt et al. 2016). The receptor was expressed using *Sf9* insect cells to produce high levels of functional protein, which was then purified using lauryl maltose neopentyl glycol (LMNG), a protein-stabilizing detergent that has been widely used in the GPCR field. This detergent has an exceptionally low critical micelle concentration (Chung et al. 2012), and it has been shown to enhance stability of monomeric membrane proteins as well as preserve the stability of multi-protein complexes (Chae et al. 2010).

Purification of the full-length Sigma1 protein was achieved by antibody affinity chromatography, allowing rapid isolation of Sigma1 in high biochemical purity (Schmidt et al. 2016). Purified protein was then crystallized using the lipidic cubic phase (LCP) method (Caffrey et al. 2012). This approach entails reconstituting the protein in a liquid crystalline lipid bilayer and performing crystallization experiments with Sigma1 embedded in this membrane throughout the crystallogenesis process. Consequently, LCP crystals typically show arrays of protein molecules in flat membrane bilayers stacked together to form the crystal lattice. In the case of Sigma1, this approach led to structures (Schmidt et al. 2016) of the receptor bound to two chemically distinct ligands: PD144418, a high-affinity, selective antagonist (Lever et al. 2014; Akunne et al. 1997), and 4-IBP, a high-affinity ligand with an incompletely understood efficacy profile (John et al. 1994). These ligands were chosen by screening a small collection of compounds with 1 nM or higher affinity in crystallization trials, with PD144418 and 4-IBP showing the largest improvements in crystal size and appearance. While Sigma1 structure agrees closely with prior pharmacological data, it shows little similarity to previous computational models (Laurini et al. 2011), highlighting the challenges of predicting membrane protein structure *ab initio*.

The overall structure of the protein is unusual, with no significant resemblance to any other protein of known structure. Contrary to the prevailing view that the receptor

possessed a two-pass transmembrane architecture, the structure reveals definitively that Sigma1 possesses only a single transmembrane domain, located at its amino terminus and encompassing residues 8 to 32. The remainder of the structure forms a single domain, with the ligand-binding site at its center. In the crystal, the receptor exists in a trimeric arrangement in which three protomers are intimately associated to form a flat triangle, with a transmembrane domain at each corner (Fig. 1 a, b). The entire membrane-proximal surface is flat and hydrophobic, and the presence of ordered lipid molecules indicates that this surface is likely buried within the membrane. The receptor thus contains structural elements typical of both transmembrane receptors and peripheral membrane proteins.

The carboxy-terminal cytosolic domain of Sigma1 comprises the bulk of the protein, encompassing residues from 33 to 223. The overall fold of this region is unlike any other protein crystallized to date, but at its core contains a cupin-like β -barrel which encloses the bound ligand (Fig. 2). The cupin fold is a conserved structural motif found in a wide variety of proteins, many of which are bacterial metalloenzymes. In most of these proteins, the barrel-like cupin fold is essential for binding to small-molecule substrates and catalytic metal ions (Dunwell et al. 2001). In most cases, these enzymes perform redox chemistry on small-molecule metabolites. In the case of Sigma1, this fold serves to envelope the ligand, occluding it entirely from solvent. The carboxy-terminal domain also contains the entire oligomerization interface, mediating the threefold non-crystallographic symmetry. Although each of the three protomers in the structure is crystallographically independent, they show no significant differences other than in orientation of the transmembrane domain. Lattice contacts are mostly mediated by these transmembrane domains, which pack in both parallel and antiparallel configurations to form the crystal.

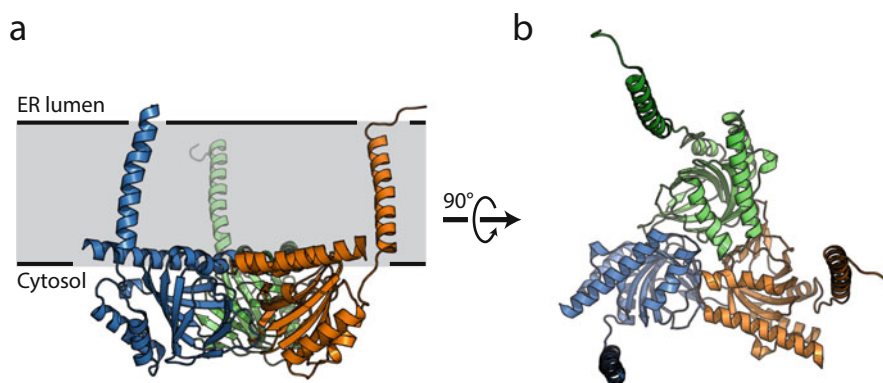


Fig. 1 Overall structure of Sigma1: The overall fold of Sigma1 is unusual, with three protomers forming a triangular structure with a single transmembrane helix at each corner. The majority of the protein is located carboxy-terminal to the transmembrane domain, on the cytoplasmic surface of the membrane. (a) The structure is shown viewed parallel to the membrane plane. (b) The triangular architecture of the receptor is apparent when viewed through the membrane plane facing the cytosol

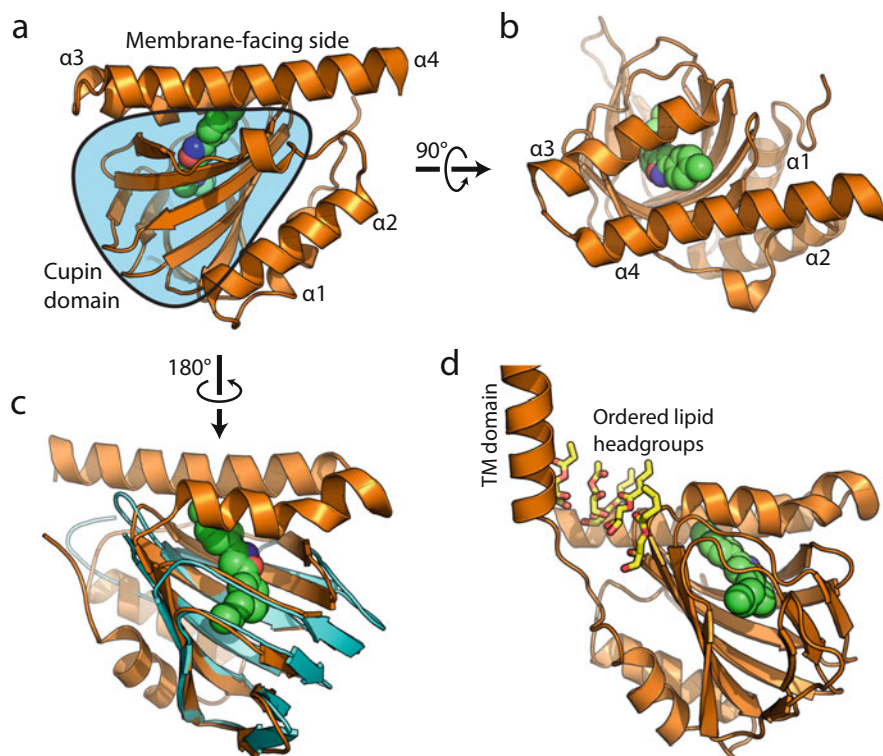


Fig. 2 Structure of the carboxy-terminal domain: The carboxy-terminal domain of Sigma1 makes up the bulk of the protein. (a) Viewed from the side (i.e., parallel to the membrane) the cupin domain at the core of the protein is shaded in blue. The bound antagonist PD144418 is shown in spheres at the center of the cupin domain. (b) Viewed from the top (membrane-facing side) the major structural elements are visible. (c) A bacterial cupin protein (light blue, PDB ID 3BCW) is superimposed on Sigma1, showing the high degree of structural conservation. (d) A cluster of ordered lipids are observed in the crystal structure at the junction of the carboxy-terminal domain and the transmembrane helix (shown in yellow sticks, oxygen atoms in red). The flexible tails of the lipids are not crystallographically resolved

3 Structural Basis for Ligand Recognition

The structures of Sigma1 bound to two drug-like ligands offer the first detailed views of ligand recognition by the receptor. The two structures are remarkably similar to one another, with few differences in ligand/receptor interactions (Schmidt et al. 2016). Previous work using site-directed mutagenesis and radioligand-binding assays identified many of the key residues that are essential for ligand-binding activity. Among these, Asp126 and Glu172 are particularly notable, as mutation of either results in a profound loss of ligand-binding activity (Seth et al. 2001). The crystal structures show

that Glu172 serves as a counterion to the protonated ligand amine, directly interacting via a hydrogen bond. Like Glu172, Asp126 is also essential for high-affinity ligand binding. Unlike Glu172 however, it does not interact directly with the ligand. Instead, Asp126 engages in a 2.6 Å hydrogen bond with Glu172, indicating that it must be protonated and resulting in overall charge neutrality in the binding pocket when the ligand is bound. With the exception of Asp126 and Glu172, the ligand-binding site is hydrophobic overall, and is largely composed of aromatic residues. Figure 3 shows the structure of the ligand-binding site, highlighting residues in contact with the bound ligand.

The solvent-occluded charge–charge interaction in the binding site closely resembles similar ligand-binding modes observed in biogenic amine GPCRs, including receptors for acetylcholine (Haga et al. 2012; Kruse et al. 2012), dopamine (Chien et al. 2010), histamine (Shimamura et al. 2011), and serotonin (Wang et al. 2013; Wacker et al. 2013). In these receptors, the conserved residue Asp3.32 (Ballesteros–Weinstein numbering (Ballesteros and Weinstein 1995)) engages in a salt bridge with the protonated ligand amine, paralleling the role of Glu172 in Sigma1. Although Sigma1 is unrelated to the aminergic GPCRs in sequence, the close parallels in binding site structure offer an explanation for the cross-reactivity of ligands like haloperidol, which binds both D₂ dopamine receptor and Sigma1 with nanomolar potency. It is possible that the similar binding modes arise from convergent evolution of biogenic amine recognition sites, which could imply that Sigma1 acts as a receptor for a neurotransmitter or similar small molecule. However, as discussed below it remains unknown if Sigma1 indeed responds to any endogenous agonist.

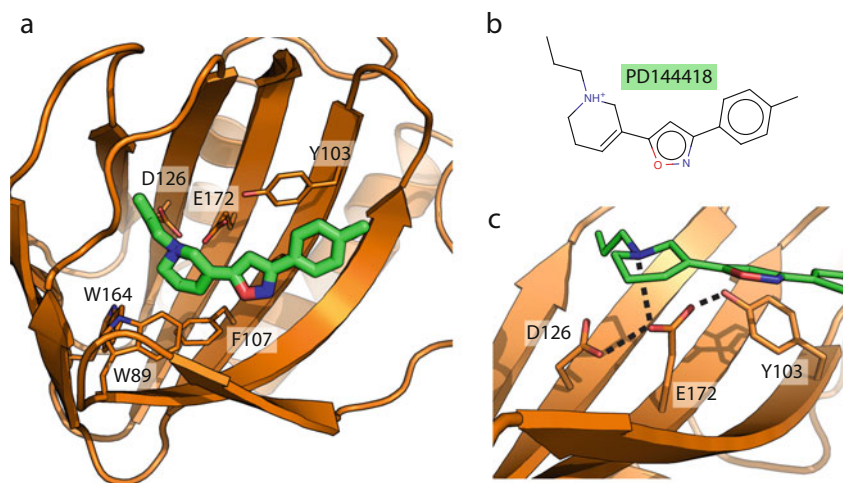


Fig. 3 Structure of the ligand-binding site: The ligand-binding site of Sigma1 is shown in two different views. (a) An overall view of the binding site reveals the largely hydrophobic contacts between the ligand and Sigma1, with the exception of a salt bridge to Glu172. (b) The chemical structure of the bound ligand PD144418. (c) A close-up of the binding site shows the extended polar network including Asp126 and Tyr103 as hydrogen-bonding partners to Glu172

Surprisingly, the ligand-binding site in both of the crystal structures is entirely occluded from solvent, offering no possible path for ligand entry or egress. This shows that the protein must be able to undergo dynamic changes to allow ligands to bind and dissociation from the receptor, but the path of ligand entry and exit remains unknown. The enclosed ligand-binding site offers a clear explanation for the very slow binding kinetics of most sigma ligands (Itzhak 1989), contrasting with opioid receptors which show rapid binding kinetics at their highly exposed ligand-binding sites (Cassel et al. 2005).

In addition to drug-like small molecules, Sigma1 has also been shown to bind to a range of lipids, including sterols like progesterone as well as sphingolipids (Ramachandran et al. 2009). Because crystallization experiments were conducted in the absence of these molecules the structural basis for their interaction with Sigma1 remains unknown. Nonetheless, a cluster of four bound monoolein lipid molecules are observed in the structure in a cleft between the transmembrane domain and the carboxy-terminal domain. This region is flanked by Gln33, Leu100, Trp121, Val177, and Leu214, and may serve as a site for lipid regulation of Sigma1 activity.

4 Oligomerization

Recent work from multiple labs has shown evidence that the Sigma1 receptor is likely to function as an oligomer, with possible regulation of oligomerization state by small-molecule ligands (Mishra et al. 2015; Gromek et al. 2014). The crystal structures further support this idea, showing an intimately associated trimer formed by the carboxy-terminal domain of each protomer. The interaction surface is extensive, involving more than 30 residues in each protomer, primarily along loops of the adjacent β -strands in the cupin domain, particularly along the cytosolic face (Fig. 4a). The residues in the oligomerization interface are largely hydrophobic, although some hydrophilic amino acids are present at the periphery of the interface. Within the oligomerization interface Trp136 is among the most extensively engaged residues, embedded deeply within a hydrophobic pocket on the adjacent protomer formed by Phe83, Ala110, Leu111, and Trp169. A hydrogen-bonding network centered on Arg119 also links adjacent protomers (Fig. 4b), as does a threefold symmetric aromatic stacking interaction among Phe191 residues from each protomer (Fig. 4c). Importantly, the oligomerization interface is highly conserved in sequence, attesting to its functional importance.

Size-exclusion experiments with multi-angle light scattering (SEC-MALS) showed that purified Sigma1 in detergent exists in a range of oligomeric states, with molecular weights ranging from at least 140 up to 400 kDa (Schmidt et al. 2016). Similarly, other biochemical and pharmacological work has shown that Sigma1 exists in a range of oligomeric states in detergents, with high-molecular-weight species being stabilized by ligands (Gromek et al. 2014). Cell-based fluorescence resonance energy transfer (FRET) studies have shown similar effects, as well as revealed agonist stabilization of low-molecular-weight species (Mishra et al. 2015). Taken together, these data indicate that oligomerization is an important feature of Sigma1 function, although the

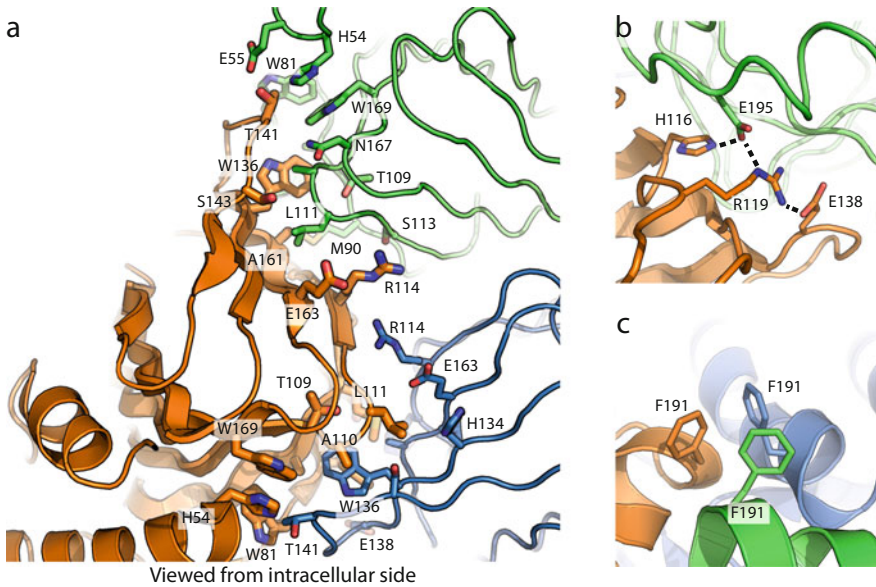


Fig. 4 Oligomerization: The interactions among Sigma1 protomers are extensive, involving dozens of amino acids primarily on the cytosolic face of the trimer. Viewed from the cytosolic face most of the oligomerization contact residues are resolved, shown as *sticks*

mechanistic details of oligomerization changes and any regulation thereof remain to be fully elucidated.

5 Implications for Sigma1 Function

While the Sigma1 receptor has been extensively studied by ligand-binding assays, the identity of the endogenous ligand, if any, remains unclear. Proposed ligands include dimethyltryptamine (Fontanilla et al. 2009) (DMT), but this has been called into question (Keiser et al. 2009) because of the much higher affinity of DMT at serotonergic receptors including the 5-HT_{2A} receptor, which binds to DMT with 100-fold higher affinity than does Sigma1. Moreover, the behavioral effects of DMT administration are abrogated in 5-HT_{2A} receptor knockout mice (Keiser et al. 2009). Given these results, the identity of any endogenous Sigma1 ligand remains unclear.

Indeed, it is possible that the term “receptor” is something of a misnomer, and Sigma1 may function in an altogether different manner than conventional receptor families. One intriguing possibility is suggested by the close sequence homology between Sigma1 and the fungal sterol isomerase *ERG2*. While it has been demonstrated that Sigma1 cannot complement *ERG2* gene deletion in yeast (Hanner et al. 1996), it remains possible that Sigma1 possesses enzymatic activity that has yet to be discovered. Alternatively, Sigma1 may be the result of evolutionary repurposing

of an enzyme to a receptor, converting an active site into a ligand-binding site for regulation of receptor function.

In addition to pharmacological research, Sigma1 has been extensively probed in cell biological studies. This work has offered insight into a wide variety of aspects of Sigma1 activity, suggesting a possible role as a multifunctional regulator of transmembrane signaling. Interactions have been reported with GPCRs (Kim et al. 2010), ion channels (Aydar et al. 2002; Balasuriya et al. 2014), and chaperone proteins (Hayashi and Su 2007), among many others (Su et al. 2016). Current structural data are insufficient to comment substantively on the nature of such interactions, but this will doubtless be an important area for future research. Importantly, the advent of structural data now allows more rational construct design and analysis for cellular work. In particular, many previous experiments used constructs designed based on incorrect topological models that identified residues 100–223 as the carboxy-terminal domain. We now know that residues 33–99 are also integral parts of this domain, contributing two out of ten beta strands and two out of four alpha helices. Accordingly, the interpretation of research that used only partial fragments of this domain may need to be reconsidered.

6 Outlook

With the advent of high-resolution structural data for Sigma1, the field is poised for new insights and reconsideration of previous models. The discovery that Sigma1 possesses only a single transmembrane domain in particular highlights the risks associated with overreliance on a single model, and should now guide informed design of modified receptor constructs in future work. Despite the important insights offered by recent structures, other key questions remain unanswered and several areas for future research are highlighted below. In the long term, a complete understanding of Sigma1 biology will require a molecular understanding of ligand binding, efficacy, and regulation of interactions with other proteins. In each respect, structural biology is likely to play a pivotal role.

6.1 Relationship to Erg2p and Enzymatic Activity

The sequencing of Sigma1 20 years ago offered the first clear connection to a protein of well-described function, revealing sequence similarity to the yeast sterol isomerase Erg2p. In fungi, this enzyme plays an essential role in ergosterol biosynthesis, catalyzing the transfer of a double bond between the C8 and C9 positions to a new site between the C8 and C7 carbons. In humans, the analogous reaction is catalyzed by the emopamil-binding protein (EBP), which is unrelated to Sigma1 and Erg2p in primary sequence (Moebius et al. 1997). The sequence similarity between Sigma1 and Erg2p implies that the latter is likely to possess a similar membrane-embedded fold. This would allow the hydrophobic lipid substrate (fecosterol) to access the

catalytic site, while the equally hydrophobic product (episterol) can then escape directly into the bilayer.

Despite their sequence similarity and conservation of the catalytic/ligand-binding glutamate (Glu174 in yeast *Erg2p*, Glu172 in human *Sigma1*), the *Sigma1* receptor fails to complement *ERG2* gene deletion in yeast (Hanner et al. 1996). This fact has been taken as evidence for a lack of *Sigma1* catalytic activity. Nonetheless, *Sigma1* has not been directly assessed for its ability to catalyze the analogous transformation in cholesterol, and it remains possible that an enzymatic activity like that of *EBP* may in fact be present. The development of robust procedures for purification of homogeneous, functional *Sigma1* should now allow straightforward assessment of this possibility in the near future.

6.2 Molecular Efficacy and Oligomerization

Many *Sigma1* ligands have been classified as agonists and antagonists on the basis of their effects on animals (Nguyen et al. 2015). However, little is known regarding the molecular basis for ligand efficacy in terms of the specific receptor conformation(s) stabilized by agonists vs. antagonists. Cellular FRET data suggest a possible role for at least some *Sigma1* antagonists in stabilizing high-molecular-weight oligomers, while certain agonists suppress oligomerization (Mishra et al. 2015). Nonetheless, the molecular mechanistic basis for these effects remains unknown. While it is increasingly apparent that oligomerization is a key aspect of *Sigma1* function, its exact role and connection to ligand efficacy are likely to be important areas of research in years to come. In particular, elucidation of distinct conformations/oligomerization states is poised to be an important area for *Sigma1* structural biology.

6.3 Interactions with Other Proteins

Sigma1 interactions with other proteins have been the subject of intense investigation for decades, with a wide range of proteins proposed as interaction partners (Su et al. 2016). Key areas for future work include validating these interactions with purified proteins, mapping sites of interaction, and determining the molecular basis for regulation of *Sigma1*/effector protein interactions. Recent advances in high-resolution electron microscopy (Liao et al. 2013) are particularly exciting, as these techniques may allow investigation of *Sigma1* complexes with high-molecular-weight putative binding partners like inositol phosphate receptors, for which electron microscopy structural data has recently become available (Fan et al. 2015).

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